U.S. Army Center for Health Promotion and Preventive Medicine

Wildlife Toxicity Assessment for PENTAERYTHRITOL TETRANITRATE (PETN)

NOVEMBER 2001

Prepared by
Health Effects Research Program
Environmental Health Risk Assessment Program

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FINAL REPORT
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Acknowledgements

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Department of the Army
U.S. Army Center for Health Promotion and Preventive Medicine

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CAS Nos. 78-11-5 **November 2001**

1. INTRODUCTION

Pentaerythritol tetranitrate (PETN) is an explosive chemical that is currently used as the primary ingredient in detonating fuses and as a component (mixed with hexahydro-1,2,5-trinitro-1,3,4-triazine) in "plastic" explosives such as *Semtex*. Structurally, PETN (Chemical Abstract Services Registry Number 78-11-5) resembles nitroglyerin, a compound whose pharmacological as well as explosive properties it shares. Thus, in the human health field, PETN and nitroglycerin are used medicinally in the treatment of angina, through their shared vasodilatory action. However, for either drug, repeated exposure can establish a sequence of tolerance, then dependence as the body adjusts to the presence of either hypotensive agent. Thus, for occupationally exposed subjects, cycles of exposure and withdrawal associated with a 5-day workweek (exposure) followed by a 2-day weekend (withdrawal) can lead to the well-recognized "Monday-morning death" of munitions employees who are exposed to these substances on a regular basis. The phenomenon arises from cardiovascular events that are triggered by unrestrained compensatory vasoconstriction, as the normally high organic nitrate levels in the body become reduced during the weekend (Abrams, 1980). The importance of PETN as an environmental contaminant is related to its distribution at and around military sites and to its potential toxicity to wildlife and other ecological receptors. This Wildlife Toxicity Assessment summarizes current knowledge of the likely harmful impacts of PETN on wildlife, emphasizing threshold doses for the onset of toxicological effects, as described in reports of experimental studies of PETN. Surveying the threshold dosimetry of the compound may point to the establishment of toxicity reference values (TRVs) that could serve as protective exposure standards for all wildlife ranging in the vicinity of affected sites. The protocol for the performance of this assessment is documented in the U.S. Army Center for Health Promotion and Preventive Medicine Technical Guide 254, the *Standard Practice for Wildlife Toxicity Reference Values* (USACHPPM 2000).

2. TOXICITY PROFILE

2.1 Literature Review

Relevant biomedical, toxicological and ecological databases were electronically searched August 23, 2000, using DIALOG to identify primary reports of studies and reviews on the toxicology of PETN. Separate searches were carried out linking the compound to either laboratory mammals, birds, reptiles and amphibians (combined) and wild mammals. All available abstracts of those articles that were selected as possibly relevant to TRV development were evaluated for relevancy. For PETN, three articles were marked for retrieval from 47 initial hits. Details of the search strategy and the results of the search are documented in Appendix A. The Defense Technical Information Center was searched for relevant U.S. Army reports. A secondary source was the National Library of Medicine's Hazardous Substances Databank (HSDB, 2000).

2.2 Environmental Fate and Transport

PETN is produced in much smaller quantities than other military explosives. Furthermore, there are few data on the compound's environmental fate or on its site-specific concentrations in environmental media. Physicochemical characteristics of PETN that may be relevant to the environmental fate and transport of the compound are listed in Table 1.

Table 1 Summary of Physical-Chemical Properties of PETN

Molecular weight	316.15
Color	white
Physical state	crystalline solid
Melting point	140 ^{o}C
Boiling point	180 ^{o}C (decomposes above 150 ^{o}C)
Odor	slight
Solubility Water	43 mg/L at 25 ^{o}C; soluble in benzene, sparingly soluble in alcohol, ether, etc.
Partition coefficients:	
Log K_{ow}	1.61
Log K_{oc}	2.25–3.24
Vapor pressure at 25 ^{o}C	1.035×10^{-10} mm Hg
Henry's Law constant at 25 ^{o}C	1.2×10^{-11} atm.m^3/mole
Conversion factors	1 ppm = 12.93 mg/m^3
	1 mg/m^3 = 0.077 ppm

Source: HSDB, 2000

Layton et al. (1987) were unable to find any information about the compound's ability to undergo photolysis but speculated that the process is unlikely to be significant since the compound absorbs little ultraviolet or visible light.

The estimated values for vapor pressure and Henry's Law constants are sufficiently low to indicate that PETN is unlikely to be released to the air as a vapor. However, aerial dispersion of the compound adhering to soil or dust particles is a possible mechanism by which PETN can be released to the atmosphere. The low water solubility coupled with the low Kow suggests that PETN is not very bioavailable to most organisms.

2.3 Summary of Mammalian Toxicity

2.3.1 Mammalian Oral Toxicity

2.3.1.1 Mammalian Oral Toxicity – Acute

Von Oettingen et al. (1944) administered a 20 mg/ml PETN solution by gavage to young female albino rats. Following a six-hour period, the entire gastrointestinal tract was removed and the contents analyzed. It was determined that only 13% of the compound was absorbed. Dogs were also given oral doses of 5 mg/kg PETN with only a gradual decrease in blood pressure with a corresponding increase in respiratory rate and minute volume reported. No details were presented. No other studies were identified.

2.3.1.2 Mammalian Oral Toxicity – Subacute

One of the few reports of studies on the toxicity of PETN in laboratory animals was that of NTP (1989), which addressed the toxicity of the compound mixed in a 1:4 ratio with D-lactose monohydrate as a stabilizer, the mixture being designated "PETN, NF." In the various phases of the study, the mixture was administered to F344 rats and B6C3F1 mice in the diet for either 14 days, 13–14 weeks, or 2 years. In the subacute phase of the study, five rats and five mice/sex/group were exposed to 0, 3100, 6200, 12500, 25000 or 50,000 mg PETN, NF/kg diet for 14 days. Based on the data in the study, these concentrations approximated to average daily doses of PETN of 0, 54, 111, 258, 502 and 909 mg/kg-day in male rats, 0, 69, 145, 272, 552 and 1087 mg/kg-day in female rats, 0, 164, 281, 556, 1255 and 2587 mg/kg-day in male mice and 0, 215, 534, 784, 1675 and 3333 mg/kg-day in female mice. However, no clinical signs nor toxicological responses were induced in any of the animals under investigation.

2.3.1.3 Mammalian Oral Toxicity – Subchronic

The same dietary concentrations of PETN, NF that were used in the 14-day study were administered to 10 rats and mice/sex/group for 13 or 14 weeks, resulting in average daily doses of 0, 43, 90, 203, 319 or 625 mg PETN/kg-day to male rats, 0, 54, 105, 240, 463 or 931 mg/kg-day to female rats, 0, 131, 319, 450, 1028 or 2163 mg/kg-day to male mice and 0, 780, 336, 694, 1406 or 3170 mg/kg-day to female

mice, as calculated from the data in the study (NTP 1989). Consistent with the results of the subacute study, few if any overtly PETN-related effects were observed among the responses, although a tendency for higher than normal relative brain and kidney weights was observed in the three high-dose and the highest dose in female rats, respectively. Nitrite was detected sporadically in the urine of some male and female rats, and a Zymbal gland adenoma was detected in a single high-dose female rat. Only female mice exposed to the 50,000 ppm group had lower mean body weights (13%) than controls. No other changes were observed. In general, however, the concept that PETN is comparatively free of toxic effects in F344 rats and B6C3F1 mice at the administered doses is reinforced by the findings of this phase of the study.

Aside from the NTP (1989) report, Kodja et al. (1995) exposed nine female New Zealand white rabbits/group for 15 weeks to 6 mg/kg-day PETN in either regular chow or chow with added cholesterol. The hypercholesterolemic effects of the cholesterol-spiked diet were marked, although the presence of PETN appeared to significantly reduce the incidence and size of consequent atherosclerotic lesions. Twenty-four hours after the completion of the feeding study, excised cross-sectional pieces of the thoracic aorta were tested for endothelial functional integrity, as indicated by their contractile response to KCl, phenylephrine and by their subsequent vasorelaxant response to acetylcholine, in each case measured by a force-displacement transducer. In general, the different diets induced only marginal effects on the contractile response of the thoracic aorta to KCl or to phenylephrine. However, PETN slightly reduced the vasorelaxing potency of acetylcholine.

2.3.1.4 Mammalian Oral Toxicity – Chronic

The 2-year component of the NTP (1989) study on PETN, NF featured the dietary administration of 0, 25,000 and 50,000 mg PETN, NF/kg chow to 50 male F344 rats/group and to both sexes of B6C3F1 mice, and 0, 6200 or 12500 mg PETN, NF/kg chow to 50 female F344 rats/group. The authors report average doses in the rats of 0, 300, and 625 mg PETN/kg-day in males, 0, 100 and 208 mg/kg-day in females, and in the mice 0, 1000 and 2025 mg/kg-day in males and 0, 1275 and 2425 mg/kg-day in females. Clinical signs were monitored twice daily, and body weights and food consumption weekly for 13 weeks, then monthly. At termination, a full suite of organs and tissues were examined at necropsy and preserved by fixation. Histopathological examinations were carried out on sections from all high-dose animals and controls.

In the rats, survival was comparable among the groups and there were no obvious clinical signs. Body weights and food consumption in PETN-receiving groups were similar to controls and there were few noteworthy necropsy findings. Mean body weights of high dose male rats were 2%-9% lower than the controls throughout the study; body weights of all other groups of rats were similar. Histopathologically important findings were restricted to the Zymbal gland, in which a small but an increased incidence in

adenomas and carcinomas was observed compared to controls. However, the observed incidence was within historical data of non-treated rats (NTP 1989). This dose-dependent change, while statistically insignificant when compared to controls (Fisher's exact test), was considered by the NTP study scientists and review board to constitute equivocal evidence of the compound's carcinogenicity in this species. However, no such response was evident among the mice, in which few if any compound-related toxicological effects of PETN, NF were evident. No non-neoplastic lesions were attributable to PETN exposure in either animal model. Mononuclear leukemia rates in male rats were significantly lower relative to treatment. Hepatocellular ademomas or carcinomas also occurred with a significant trend in female mice.

Von Oettingen et al. (1944) also reported a lack of observed effects in a 1-year study in an unspecified strain of young albino rats. Two groups of 45 rats were given either a control diet or one equivalent to 2 mg/kg-d PETN. Food consumption was measured daily and animals were weighed weekly. Blood parameters were evaluated at monthly intervals. Other than a tendency for PETN-exposed animals showing monthly blood parameters having slightly higher hemoglobin and erythrocytes values, no other effects were seen. Additional evaluations included a histopathological examination of the brain, heart, lungs, adrenals, liver, spleen, kidneys, testis, and femur.

2.3.1.5 Studies Relevant to Mammalian TRV Development for Oral Exposures

In general, there is a paucity of experimental data that suggest adverse effects from oral exposure to PETN. However, given the structural similarities corroboration of PETN toxicity with that of nitroglycerin and erythritol-tetranitrate, it is likely that the mode of action is similar. Comparisons of these data also suggest that PETN is less bioavailable (von Oettingen et al. 1944).

The incidences of neoplasms associated with the Zymbal gland were within the bounds of historical results and only apparent in rats from the 104-week study. Moreover, there were no differences in the survival of the rats and mice at the end of the 104-week studies and any ecological impact of cancer in senescent animals is very uncertain and of questionable relevance. In addition, while few reports of studies on the acute lethality of the compound were found, most of the experimental evidence from the NTP (1989) report suggests the benign nature of the compound when administered to experimental animals (Table 2). Thus, when PETN, NF was administered to F344 rats and B6C3F1 mice in dietary concentrations that were at the maximum practical limit (5% w/w), few if any overtly toxicological responses were observed during exposure periods extending up to 104 weeks. Among the few possibly compound-related effects of PETN were the trends toward increased relative kidney weights that achieved statistical significance compared to controls in high-dose female rats after 13 weeks, and the dose-dependent increase in combined adenomas and carcinomas of the Zymbal gland in male and female rats exposed for 2 years. However, the marginal nature of each of these individual responses raises

questions as to whether these findings are incidental or truly compound-related. For example, supporting evidence of the compound's carcinogenicity might be expected to arise from mutagenicity/genotoxicity studies. However, in this instance also, the evidence is equivocal, since the compound has been found to be negative for gene reversion in the Ames test (Mortelmans et al., 1986), negative for the induction of chromosomal aberrations in Chinese hamster ovary cells, but positive for the induction of sister chromatid exchanges in the same experimental system (NTP, 1989).

Taken together these findings speak to the comparatively benign nature of the compound in experimental animals, though within the context of a limited information base. Both NTP studies (14 and 104 week studies in rats and mice) were conducted, reported, and recorded appropriately and are of sufficient quality to be deemed relevant for TRV derivation.

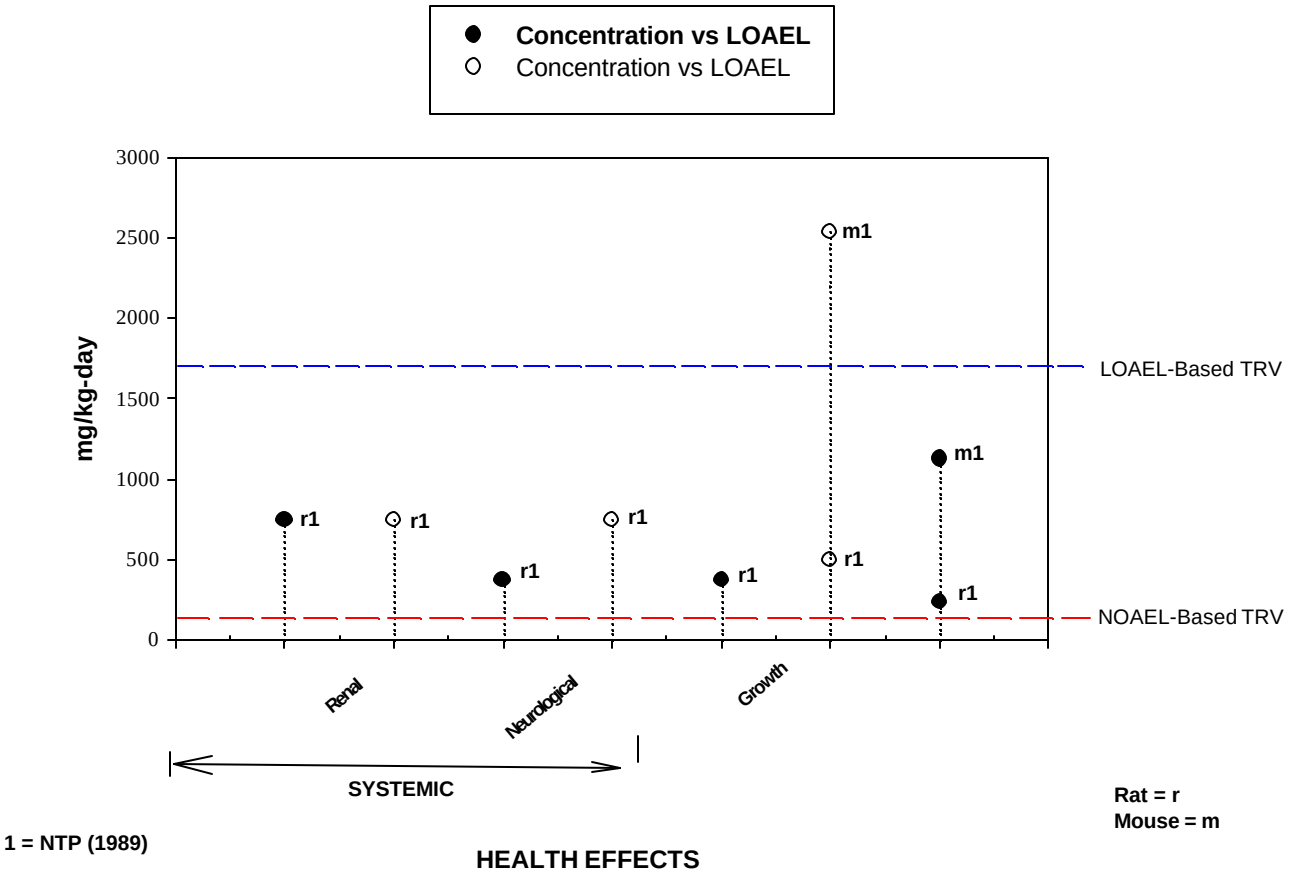
Table 2. Summary of Relevant Mammalian Data for TRV Derivation

Study	Test Organism	Test Duration	Test Results		
			NOAEL (mg/kg/d)	LOAEL (mg/kg/d)	Effects Observed at the LOAEL
NTP (1989)	Rat (F344)	14-w	370 (f) 495 (m)	745 (f) ND (m)	Increase in relative brain and kidney weight in females.
		104-w	240 (m) 166 (f)	500 (m)* ND	Males had 2-9% lower body weight than controls.
NTP (1989)	Mouse (B6C3F1)	14-w	1125 (f) 1730 (m)	2535 (f) ND (m)	Females had 13% lower body weight than controls.
		104-w	1620 (m) 1940 (f)	ND	No adverse effects in mice were observed.
von Oettingen et al (1944)	Dog	Not reported	5	ND	Mild changes in blood pressure and respiratory rate. Secondary source: not used in TRV derivation.
	Rat (strain unspecified)	1-year	2	ND	No effects observed. Secondary source: not used in TRV derivation.

***** The incidences of Zymbal gland neoplasms and non neoplasms were determined to be of "equivocal significance" by the authors on the ability of PETN to be a causitative agent. No differences in survival were seen in any of the dose groups (NTP 1989).

ND = not determined

PETN HEALTH EFFECTS TO MAMMALS



2.3.2 Mammalian Oral Toxicity – Other

No other data relevant to oral exposures for mammals were found.

2.3.3 Mammalian Inhalation Toxicity

No inhalation studies conducted using animals were found. Von Oettingen et al (1944) reported that PETN is absorbed through the lungs from observations of a fall in blood pressure and a rise in venous and spinal pressures following insufflation of 100 mg PETN in the lower trachea of dogs.

2.3.4 Mammalian Dermal Toxicity

No dermal studies conducted using animals were found. However, a study where PETN was mixed with acetone and was rubbed onto the hands of a human volunteer resulted essentially in the recovery of the entire amount of compound through washing (von Oettingen et al. 1944). This suggests that the probability for dermal absorption is relatively low.

2.4 Summary of Avian Toxicology

Toxicological data for the effects of PETN in avian species was not located. Ecotoxicological research on the effects of this compound in birds is recommended.

2.5 Summary of Amphibian Toxicology

Toxicological data for the effects of PETN in amphibian species was not located. Ecotoxicological research on the effects of this compound in amphibians is recommended.

2.6 Summary of Reptilian Toxicology

Toxicological data for the effects of 1,3,5-TNB in reptilian species was not located. Ecotoxicological research on the effects of this compound in reptiles is recommended.

3. RECOMMENDED TOXICITY REFERENCE VALUES

3.1 Toxicity Reference Values for Mammals[1]

3.1.1 TRVs for Ingestion Exposures for the Class Mammalia

The suite of studies reported by NTP (1989) are comprehensive and of good quality. The NTP (1989) report contains subacute, subchronic, and chronic study information for mice and rats. Extremely high doses were used in an effort to bound the effects data. However, no corroborative effects were observed in either target or magnitude of effect. For example, kidney and brain weights were increased in the high dose for female rats only in the 14-week study, yet there was no other information that suggested that adverse effects were observed relative to the kidney and brain function (i.e., histopathology results revealed no effect), nor did the chronic study show the same effects. Male rats in the high dose group in the 104-week study showed a 2-9% lower body mass than controls, yet these effects were not seen in females or either sex in the 14-week study.

The mouse data were also equivocal. Only the 14-week study showed a significant change in body weight (13%, females only), yet no adverse effects were seen in either sex for the 104-week study. Altogether, these data suggest the likelihood of false positive data rather than a compound-related effect. In addition, the data reported in von Oettingen (1944) also suggest no adverse effects from prolonged oral exposure to PETN.

Information is available from three species and two orders (Carnivora and Rodentia). However, only one chronic LOAEL of questionable relevance is available. Therefore, the NOAEL-LOAEL approach was approximated. Since the highest dose tested in rats approximated 166 and 500 mg/kg-d for female and male rats, respectively, the TRVs were calculated from the female NOAEL (166 mg/kg-d) according to TG254 (USACHPPM 2000). The NOAEL-based TRV for mammals is therefore 170 mg/kg-d and the LOAEL-based TRV for mammals is 1700 mg/kg-d (through the application of a 10-fold uncertainty factor. Since the LOAEL observations do not provide clear indications of an adverse effect, and that only rodents are represented, it is given a LOW confidence rating. However, since no indications of adverse effects were seen at the NOAEL, the NOAEL-based TRV was given a MEDIUM level of confidence. Based on these data it is believed that these levels should be protective of species of mammals.

[1] TRVs are for screening purposes only and are not intended to be predictors of effects in field situations. Site specific conditions may justify adjustments of these values based on toxicity information relevant to specific assessment endpoints.

Table 3. Selected Ingestion TRVs for the Class Mammalia

TRV	Dose	Confidence
NOAEL-based	170 mg/kg/d	Medium
LOAEL-based	1700 mg/kg-d	Low

3.1.1.1 TRVs for Ingestion Exposures for Mammalian Foraging Guilds

TRVs specific to particular guild associations (e.g., small herbivorous mammals) have not yet been derived. However, these data are representative of omnivorous rodents, and may be considered as such. More data for other species would be needed to derive values for other foraging guilds.

3.1.2 TRVs for Inhalation Exposures for the Class Mammalia

Not available at this time.

3.1.3 TRVs for Dermal Exposures for the Class Mammalia

Not available at this time.

3.2 Toxicity Reference Values for Birds

Not available at this time.

4. IMPORTANT RESEARCH NEEDS

Clear adverse effects from exposure to PETN appear to be lacking. Given the concentrations used in some of the studies reported herein, it is unlikely that further research using traditional laboratory animals would yield different results. However, testing in other mammalian, amphibian, avian, and reptilian species is needed to determine if these observations are different from that of laboratory mammalian models.

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APPENDIX A

LITERATURE REVIEW

The following files were searched in Dialog:

File 155 MEDLINE; File 156, TOXLINE, File 5 BIOSIS, File 10 AGRICOLA, File 203 AGRIS, File 399 Chemical Abstracts, File 337 CHEMTOX, File 77 Conference Papers Index, File 35 Dissertation Abstracts, File 40 ENVIRONLINE, File 68 Environmental Bibliography, File 76 Life Sciences Collection, File 41 Pollution Abstracts, File 336 RTECS, File 370 Science, File 143 Wilson Biological & Agricultural Index, File 185 Zoological Record, File 6 NTIS, File 50 CAB, File 144 PASCAL, File 34 SCISEARCH.

The search strategy for **Amphibians & Reptiles**:

- Chemical name, synonyms, CAS numbers
- AND (amphibi? or frog or frogs or salamander? or newt or newts or toad? or reptil? or crocodil? or alligator? or caiman? snake? or lizard? or turtle? or tortoise? or terrapin?)
- RD (reduce duplicates)

The search strategy for **Birds:**

- Chemical name, synonyms, CAS numbers
- And chicken? or duck or duckling? or ducks or mallard? or quail? or (japanese()quail?) or coturnix or (gallus()domesticus) or platyrhyn? or anas or aves or avian or bird? or (song()bird?) or bobwhite? or (water()bird) or (water()fowl)
- RD

The search strategy for **Laboratory Mammals:**

- Chemical name, synonyms, CAS numbers
- AND (rat or rats or mice or mouse or hamster? or (guinea()pig?) or rabbit? or monkey?)
- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))
- NOT (human? or culture? or subcutaneous or vitro or gene or inject? or tumo? or inhalation or carcin? or cancer?)/ti,de
- NOT ((meeting()poster) or (meeting()abstract))
- NOT (patient? or cohort? or worker? or child? or infant? or women or men or occupational)
- RD

The search strategy for **Wild Mammals:**

- Chemical name, synonyms, CAS numbers

- And(didelphidae or opossum? or soric idae or shrew? Or talpidae or armadillo? or dasypodidae or ochotonidae or leporidae)or canidae or ursidae or procyonidae or mustelidae or felidae or cat or cats or dog or dogs or bear or bears or weasel? or skunk? or marten or martens or badger? or ferret? or mink? Or aplodontidae or beaver? or sciuridae or geomyidae or heteromyidae or castoridae or equidae or suidae or dicotylidae or cervidae or antilocapridae or bovidae arvicolinae or mycocastoridae or dipodidae or erethizontidae or sigmodon? or (harvest()mice) or (harvest()mouse) or microtus or peromyscus or reithrodontomys or onychomys or vole or voles or lemming?

- AND (reproduc? or diet or dietary or systemic or development? or histolog? or growth or neurological or behav? or mortal? or lethal? or surviv? or (drinking()water))

- RD

All abstracts from the DIALOG search were reviewed and encoded in ProCite. When the search retrieved an appreciable number of hits, *keywords in context* were reviewed to minimize costs before any abstracts were downloaded (Tier 1). However, when only a limited number of studies were identified by the search, the abstracts were downloaded at the time of the search (Tier 2).

As noted in Section 2.1, 47 hits on PETN were obtained in the initial search, all of which were sele cted for abstract evaluation. Three of these articles and reviews were retrieved for this survey.